The unbinding transition of mixed fluid membranes

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Abstract. – A phenomenological model for the unbinding transition of multi-component fluid membranes is proposed, where the unbinding transition is described using a theory analogous to Flory-Huggins theory for polymers. The coupling between the lateral phase separation of inclusion molecules and the membrane-substrate distance explains the phase coexistence between two unbound phases as observed in recent experiments by Marx *et al.* [Phys. Rev. Lett. **88**, 138102 (2002)]. Bellow a critical end-point temperature, we find that the unbinding transition becomes first-order for multi-component membranes.

Introduction. – Adhesion of membranes and vesicles is responsible for cell-cell adhesion which plays an important role in all multicellular organisms. In general, bio-adhesion is governed by the interplay of a variety of generic and specific interactions [1]. The latter interaction acts between complementary pairs of proteins such as ligand and receptor, or antibody and antigen. Well-studied examples of such coupled systems are biotin-avidin complex [2], or selectins and their sugar ligands [3].

The problem of adhesion of multi-component membranes is intimately related to that of domain formation. Experimentally, adhesion-induced lateral phase separation has been observed for various systems [4–6], and it was reported that adhesion molecules aggregate spontaneously and form tight adhering domains. From the theoretical point of view, this problem has been considered in [7] using a phenomenological model, where the inter-membrane distance is coupled to the concentration of sticker molecules on the two adhering membranes. In a different approach, a lattice model for a multi-component membrane in contact with another substrate was proposed [8], and was extended using detailed Monte Carlo simulations [9, 10]. More recently, a work combining these two approaches has been published [11].

In a recent experiment by Marx *et al.* [12], the role of a long-range repulsion due to thermal fluctuations (Helfrich repulsion) of the adhering membranes has been addressed. By using Reflection Interference Contrast Microscopy [4], a multi-component bilayer membrane

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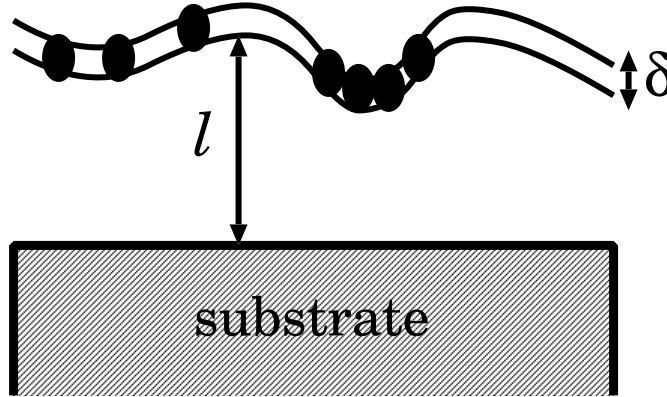(**) E-mail: `andelman@post.tau.ac.il`

Fig. 1 – A mixed fluid membrane adhering to a substrate. Black filled ovals indicate inclusions such as proteins or lipopolymers. The height of the lower membrane leaflet from the substrate is denoted by ℓ. The membrane thickness is δ.

with added lipopolymers (modified DOPE lipid with polyethylenoxide) and cholesterol is examined in the vicinity of an attractive substrate. Analyzing the probability distribution of the membrane-substrate spacing for various multi-component membranes, a phase separation between distinct lipopolymer-poor and lipopolymer-rich states was found, where both states are *unbound* from the substrate. Although this phenomena is dynamic in nature, the authors regard it as a lateral phase separation induced by the Helfrich repulsion. However a clear physical description for the appearance of such a phase coexistence has not yet been given. Moreover, a *first-order* unbinding transition scenario is required to account for the multiple time scales in the time series of multi-component membrane fluctuations [12].

In this Letter, we propose a simple phenomenological model for multi-component (mixed) fluid membranes which can undergo simultaneously a lateral phase separation and an unbinding transition. The model relies on the coupling between inclusion concentration and membrane-substrate spacing. The lateral phase separation of inclusions affects the second virial coefficient of the unbinding transition, and is taken into account in analogy to Flory-Huggins theory for polymers [13]. The proposed model is successful in describing the phase separation between two unbound states as mentioned above. Furthermore, depending on the interaction strength or the temperature, the model exhibits phase coexistence between two bound phases, or between a bound and an unbound phase. The latter case provides support to the first-order nature of the unbinding transition, as is anticipated in experiments [12].

Unbinding transition. – Fluid membranes in a lamellar stack or close to a substrate experience steric repulsion arising from their reduced undulation entropy due to the confinement effect. Helfrich showed that the corresponding interaction energy per unit area is given by [14]

$$v_{\mathrm{s}}(\ell) = \frac{b(k_{\mathrm{B}}T)^2}{\kappa \ell^2}, \tag{1}$$

where k_{B} is the Boltzmann constant, T the temperature, κ the bending rigidity of the membrane (of thickness δ), and ℓ the average height of the lower membrane lipid leaflet from the substrate (see fig. 1). The numerical prefactor b was calculated as $b = 3\pi^2/128$ in [14] but its value is still debatable in the literature [15]. The combination of the above steric repulsion and other direct microscopic (van der Waals, electric, hydration) interactions determines

whether the membrane binds to the substrate or unbinds. Using functional renormalization-group techniques, Lipowsky and Leibler showed that this unbinding transition is *second-order*. The average spacing ℓ diverges as the strength of the attractive van der Waals interaction W (known as the Hamaker constant) approaches a critical value from above, i.e., $\ell \sim (W - W_c)^{-\psi}$ with $\psi \approx 1.00$, where W_c is the critical strength of Hamaker constant [16, 17]. It should be emphasized, however, that a simple superposition of the Helfrich repulsion, eq. (1), and other direct interactions gives an incorrect (first-order) description of this unbinding transition. This failure is linked to the fact that eq. (1) was originally derived under the assumption that membranes do not interact through any other forces.

Subsequently, a much simpler theory for the unbinding transition was considered by Milner and Roux [18], and it is briefly reviewed here. Following the spirit of Flory-Huggins theory for polymers [13], the Helfrich estimate of the entropy is taken into account accurately, whereas the other interactions are approximately incorporated via a second virial term. The resulting free energy per unit area of the membrane is [19]

$$f(w) = -k_B T \chi \delta w^2 + \frac{b(k_B T)^2}{\kappa \delta^2} w^3, \tag{2}$$

where $w = \delta/\ell \geq 0$ cannot be negative. In the above, χ is a second virial coefficient describing the correction to the hard-wall repulsive interaction represented by the cubic term in w. This approach is justified since the perturbative effect of the direct interactions is included through a virial expansion rather than the simple superposition of interactions.

Consider a membrane patch of volume $\nu = a^2 \delta$, where $a = \sqrt{\kappa/k_B T}\delta$ is the in-plane cutoff. When the enthalpic interactions between the membrane patch and the substrate is small, χ is given by

$$\chi = -\frac{1}{2\nu^2} \int d^3 r \left(1 - \exp[-V_\nu(r)/k_B T]\right), \tag{3}$$

where $V_\nu(r)$ is the direct interaction energy, and the integral is limited to positions such that the membrane patches do not overlap. The free energy (2) has a continuous second-order transition at $\chi = 0$ between a bound state ($w > 0$) for $\chi > 0$ and an unbound state ($w = 0$) for $\chi < 0$. Because Milner and Roux model predicted that $\chi \sim (W - W_c)$, eq. (2) is also successful in describing the critical exponent $\psi = 1$ [18].

Unbinding transitions of mixed membranes. – So far, the unbinding transition of a single-component membrane has been discussed. In the case of multi-component membranes, the lateral phase separation affects the direct interactions between the membrane and the substrate, and hence their unbinding behavior. For simplicity, we consider a two-component membrane adhering to a substrate as in fig. 1. The overall membrane state is characterized by the membrane average distance ℓ from the substrate. The internal degree of freedom, on the other hand, corresponds to the membrane composition. In addition to the lipid component that is the main building block of the membrane, we introduce a second component or an "inclusion" representing additional lipids, proteins, cholesterol or lipopolymers also residing on the membrane. Let the concentration of these inclusions be denoted by Φ ($0 \leq \Phi \leq 1$). Here we discuss the case in which the interaction between two inclusions is attractive leading to a condensation transition. Then, below a certain temperature, the mixed membrane undergoes a first-order transition, where an inclusion-poor phase coexists with an inclusion-rich phase as observed experimentally [20]. The first-order transition terminates at a critical point, having a critical concentration Φ_c. The concentration difference is defined as $\phi = \Phi - \Phi_c$.

Using the "Flory-Huggins theory" of the unbinding transition mentioned above, and taking into account the lateral phase separation of inclusions close to the critical point, we propose

the following free energy expression of a mixed membrane undergoing an unbinding transition:

$$f(\phi, w) = -\mu\phi + \frac{1}{2}t\phi^2 + \frac{1}{4}\phi^4 - \chi w^2 + \frac{1}{2}w^3 + \phi w^2, \tag{4}$$

with the constraint $w \geq 0$. All energy terms have been scaled by $2b(k_BT)^2/\kappa\delta^2$, and have units of energy per unit area. The first three terms in eq. (4) depend only on ϕ and correspond to the Landau free energy of a two-component membrane undergoing a lateral inclusion-lipid phase separation; μ is the chemical potential and t the reduced temperature. The quartic coefficient has been chosen to be positive and is arbitrary set to $\frac{1}{4}$ without loss of generality. The next two terms depend only on w, and represent the unbinding transition of the membrane (see eq. (2)). The last term is the lowest order coupling term between ϕ and w allowed by symmetry with a coefficient that has been set to unity without loss of generality. The physical meaning of this term is as follows. When the mixed membrane is quenched into a two-phase region, an inclusion-poor phase ($\phi < 0$) coexists with an inclusion-rich phase ($\phi > 0$). This can lead to different direct interactions V_ν and hence different second virial coefficients χ for each of the domains. We model this situation by considering an effective second virial term $-\chi_{\text{eff}}w^2 = -(\chi - \phi)w^2$, which leads to the proposed coupling term ϕw^2. Notice that this term being linear in ϕ changes its sign for $\phi \to -\phi$.

In addition, a linear term in w, arising from an external force on the membrane such as pressure or gravity, can also appear in eq. (4). In the presence of such a term, the unbinding transition becomes first-order even for a single-component membrane [18]. However, this situation will not be further considered here. Another important assumption is that the phase separation of the inclusion molecules does not affect the homogeneous bending rigidity of the membrane κ. In other words, the direct microscopic (van der Waals, electrostatic, hydration) interactions are modified by the phase separation, whereas the steric interaction remains unchanged. Hence our model should be distinguished from the one in [21], where it was shown that the coupling between membrane curvature and inclusion concentration leads to lateral phase separation.

Minimizing the free energy (4) with respect to w gives $w = 0$ (unbound) for $\chi < \phi$ and $w = \frac{4}{3}(\chi - \phi) > 0$ (bound) for $\chi > \phi$. By substituting back the value of w into eq. (4), the free energy as a function of ϕ only becomes

$$f(\phi) = -\mu\phi + \frac{1}{2}t\phi^2 + \frac{1}{4}\phi^4 \qquad \text{for} \quad \phi > \chi \tag{5}$$

$$f(\phi) = -\frac{16}{27}\chi^3 + \left(\frac{16}{9}\chi^2 - \mu\right)\phi + \left(\frac{1}{2}t - \frac{16}{9}\chi\right)\phi^2 + \frac{16}{27}\phi^3 + \frac{1}{4}\phi^4 \qquad \text{for} \quad \phi < \chi \tag{6}$$

depending on the value of χ. This free energy is continuous at $\phi = \chi$. We next minimize this free energy with respect to ϕ to find the equation of state. The two-phase region is obtained by the Maxwell construction, and the whole phase diagram is calculated numerically.

Phase behavior. – The phase behavior of the above model depends on the value of χ. For $\chi > -\frac{8}{27}$, a second-order transition line (critical line) where $\phi = \chi$, separates the unbound phase ($w = 0$) from the bound one ($w > 0$), as shown in fig. 2 for $\chi = 0.5$. The critical line ends at a critical end-point (CEP) on the first-order transition line. The first-order transition line itself ends at an ordinary critical point (CP) corresponding to a liquid/vapor-type CP between two bound phases (B1+B2). Below the CEP temperature, the first-order line separates a bound phase from an unbound phase (B1+U).

The critical point between the two bound phases is given by $t_c = \frac{32}{9}(\chi + \frac{8}{27})$ and $\phi_c = -\frac{16}{27}$. Hence, the critical temperature is increased from $t_c = 0$ to $t_c \approx 2.83$ for $\chi = 0.5$ due to the
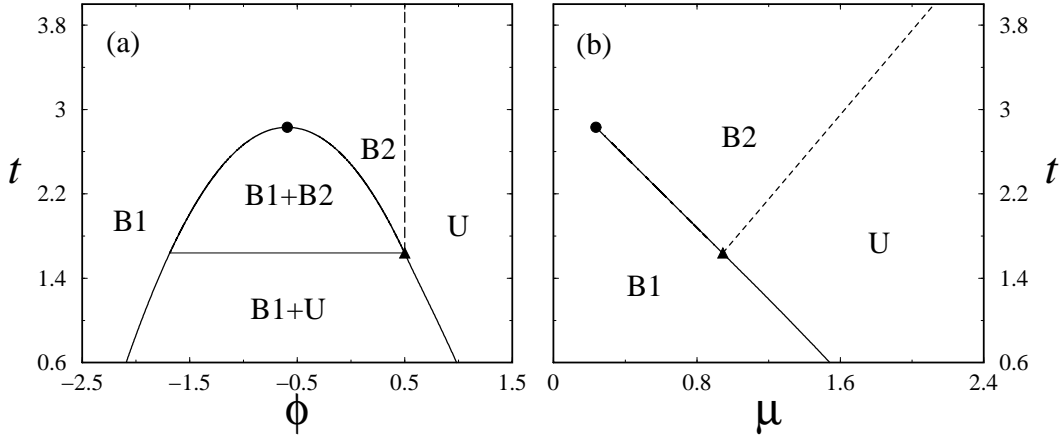
Fig. 2 – The phase diagrams for $\chi = 0.5$ as a function of (a) inclusion concentration ϕ and reduced temperature t, and (b) inclusion chemical potential μ and reduced temperature t. The continuous line is a first-order line, whereas the dashed line is a second-order one. The critical point (CP) and the critical end-point (CEP) are indicated by a filled circle and a triangle, respectively. The bound ($w > 0$) and the unbound ($w = 0$) phases are denoted as B and U, respectively. Below the critical point, there is a coexistence region either of the two bound phases (B1+B2), or a bound and an unbound phases (B1+U).

coupling between inclusion concentration ϕ and membrane-substrate distance $\ell = \delta/w$. In other words, the phase separation is *enhanced* by the adhesion for $\chi > -\frac{8}{27}$. This result is in accordance with the previous theoretical models [7, 9, 10]. Between the CEP and the CP of the two-phase region (B1+B2), the two coexisting values of ϕ lead to different membrane-substrate distances given by $\ell = \frac{3}{4}\delta(\chi - \phi)^{-1}$. Since the ℓ value for B1 is smaller than that for B2, the phases B1 and B2 correspond to "tight" and "loose" bound phases, respectively, as discussed in [7]. We see that ℓ diverges as $(\chi - \phi)^{-1}$ at $\phi = \chi$, and $w = \delta/\ell$ increases continuously from zero.

In fact, even the very simple free energy (4) leads to a rich topology of the phase diagram. In addition to the coexistence between two bound phases (B1+B2), a new two-phase region between two unbound phases (U1+U2) appears for $-\frac{32}{81} < \chi < -\frac{8}{27}$. As an example, fig. 3 gives the phase diagram for $\chi = -0.3$. In this case, two different critical points are found: one at $t_c = \frac{32}{9}(\chi + \frac{8}{27})$, $\phi_c = -\frac{16}{27}$ as before, and the other at $t_c = 0$, $\phi_c = 0$. Hence the phase separation is not necessarily enhanced. There is also a triple point (Tr) at which two bound phases and one unbound phase coexist (B1+B2+U2). Below the triple point temperature, there is a region of two-phase coexistence between a bound and an unbound phase (B1+U2).

For $\chi < -\frac{32}{81}$, a similar (ϕ, t) phase diagram as in fig. 2(a) is obtained with the critical line being located at $\phi < \phi_c = 0$ (not shown as a graph). Below the critical point $t_c = 0$, $\phi_c = 0$, two unbound phases coexist until the CEP temperature. Then a region of two-phase coexistence between a bound and an unbound phase appears below the CEP.

As mentioned before, the coexistence between two unbound states has been observed experimentally for mixed membranes with added lipopolymers and cholesterol [12]. For the case of fixed cholesterol content, distinct lipopolymer-poor and lipopolymer-rich states coexist, and both states are found in experiments to be unbound. Our model predicts that such a coexistence appears for $\chi < -\frac{8}{27}$. Note also that the unbinding transition becomes first-
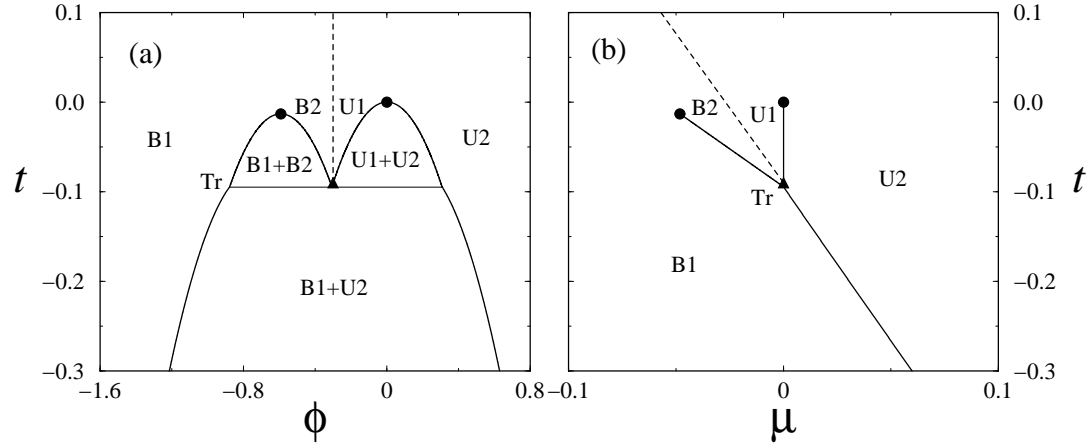
Fig. 3 – The phase diagram for $\chi = -0.3$ as a function of (a) inclusion concentration ϕ and reduced temperature t, and (b) inclusion chemical potential μ and reduced temperature t. Same notation of different lines and symbols is used as in fig. 2. In addition to the critical end-point (denoted by a filled triangle), there are two critical points (denoted by filled circles) and a triple point denoted by Tr. In (b), the CEP and Tr are two distinct but very close points, although it is hard to see it on the figure.

order below the CEP or Tr, for any value of χ. This is in accordance with the experimental prediction [12]. However, the first-order and the second-order lines are connected by a CEP and not by a tricritical point.

The present results can be compared with those obtained in other theoretical works. In our previous phenomenological model for the adhesion-induced phase separation [7], we have considered only the phase coexistence between two bound phases, and the unbinding behavior of the membrane was not taken into account. In that work we showed that the phase separation between two bound phases is always enhanced by adhesion, whereas in the present model, it can either be enhanced or suppressed, depending on χ. Somewhat similar phase diagrams to ours have been obtained in [9,10] by a mean-field treatment of a lattice model. In those works, the topology of the phase diagrams depends on several quantities such as the sticker binding energy, the potential range, or the strength of the *cis*-interaction between the stickers. Some of their phase diagrams also include a two-phase region where bound and unbound phases coexist. However, the coexistence between two unbound phases has not been predicted within the lattice model [9,10].

Extensions of the model. – We finally discuss two possible extensions of our model. The first situation deals with membranes having finite tension σ. Since the tension strongly suppresses the out-of-plane fluctuations of the membranes, the steric interaction is weakened. A self-consistent calculation of the Helfrich repulsion under tension is given by [22]

$$v_{\rm s}(\ell;\sigma) = \frac{b(k_{\rm B}T)^2}{\kappa \ell^2} \left[\frac{\ell/\lambda}{\sinh(\ell/\lambda)} \right]^2 , \tag{7}$$

where $\lambda = (2k_{\rm B}T/\pi\sigma)^{1/2}$. Following the argument in [23], the generalized free energy for the mixed membranes under tension can be obtained by replacing the term $w^2/2$ in eq. (4) with

$x^2 w^3/(2\sinh^2 x)$, where $x = \ell/\lambda$. In general, tension induces binding of membranes, in cases where membranes unbind in the *absence* of tension. Hence, for $x \gg 1$ (large tension), one expects that the region of bound phases dominates most of the phase diagram.

Another interesting case is the adhesion between two fluctuating mixed membranes. Symmetry consideration with respect to the exchange of the two membranes gives the allowed coupling terms between the inclusion concentration on each of the membranes and the distance between them. A rich phase behavior is expected when the average inclusion concentration on the two membranes is not identical [7].

Summary. – We have proposed a phenomenological model for the unbinding transition of two-component fluid membranes. The coupling between lateral phase separation and the membrane-substrate distance provides a rich phase behavior. Our model is successful in describing the first-order nature of the unbinding transition in multi-component membranes, as well as the phase separation between two unbound phases. It may provide a theoretical explanation for the experimental observations made by Marx *et al.* [12].

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REFERENCES

[1] Lipowsky R. and Sackmann E. (Editors), *Structure and Dynamics of Membranes*, Vol. **1B** (Elsevier, Amsterdam) 1995.
[2] Leckband D. E., Israelachvili J. N., Schmitt F.-J. and Knoll W., *Science*, **255** (1992) 1419.
[3] Zuckerman D. and Bruinsma R., *Phys. Rev. Lett.*, **74** (1995) 3900.
[4] Albersdörfer A., Feder T. and Sackmann E., *Biophys. J.*, **73** (1997) 245.
[5] Nardi J., Feder T., Bruinsma R. and Sackmann E., *Europhys. Lett.*, **37** (1997) 371.
[6] Bruinsma R., Behrisch A. and Sackmann E., *Phys. Rev. E*, **61** (2000) 4253.
[7] Komura S. and Andelman D., *Eur. Phys. J. E*, **3** (2000) 259.
[8] Lipowsky R., *Phys. Rev. Lett.*, **77** (1996) 1652.
[9] Weikl T. R., Netz R. R. and Lipowsky R., *Phys. Rev. E*, **62** (2000) R45.
[10] Weikl T. R. and Lipowsky R., *Phys. Rev. E*, **64** (2001) 011903.
[11] Weikl T. R., Andelman D., Komura S. and Lipowsky R., *Eur. Phys. J. E*, **??** (????) ??.
[12] Marx S., Schilling J., Sackmann E. and Bruinsma R., *Phys. Rev. Lett.*, **88** (2002) 138102.
[13] de Gennes P.-G., *Scaling Concepts in Polymer Physics* (Cornel UP, Ithaca) 1979, p. 69.
[14] Helfrich W., *Z. Naturforsch.*, **33a** (1978) 305.
[15] Sornette D. and Ostrowsky N., *Micelles, Membranes, Microemulsions, and Monolayers*, edited by Gelbart W. M., Ben-Shaul A. and Roux D. (Springer-Verlag, New York) 1994, p. 251.
[16] Lipowsky R. and Leibler S., *Phys. Rev. Lett.*, **56** (1986) 2541.
[17] Leibler S. and Lipowsky R., *Phys. Rev. B*, **35** (1987) 7004.
[18] Milner S. T. and Roux D., *J. Phys. I France*, **2** (1992) 1741.
[19] In [18], the free energy per unit volume is given since they consider a stack of membranes. Here we multiplied the membrane thickness δ to obtain the free energy per unit area of the membrane.
[20] Keller S. L., Pitcher III W. H., Huestis W. H. and McConnell H. M., *Phys. Rev. Lett.*, **81** (1998) 5019.
[21] Netz R. and Pincus P., *Phys. Rev. E*, **52** (1995) 4114.
[22] Seifert U., *Phys. Rev. Lett.*, **74** (1995) 5060.
[23] Diamant H. and Cates M. E., *Eur. Phys. J. E*, **4** (2001) 223.